BIOCHEMICAL CHANGES IN SCROTAL AND CRYPTORCHID RAT TESTIS DURING POSTNATAL MATURATION

By

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CHAPTER I

INTRODUCTION

The development of complete spermatogenesis is one of the most significant physiological changes that takes place during the life of the male. Although the histological changes that take place in the testis are well documented, a search of the literature indicates that little is known concerning the accompanying biochemical changes.

The object of this thesis was to lay the groundwork for more definitive studies on the maturation process by providing information on the levels of biochemical constituents in the scrotal versus cryptorchid testis of the albino rat during postnatal development. The constituents studied were lipids, carbohydrates, and proteins. Much of the recent literature concerning the chemistry of spermatozoa and seminal fluid was found to deal with lipids. Because of this present interest in the lipid chemistry of seminal components, the lipid changes were studied in greater detail than were the carbohydrate and protein changes. This mode of investigation should give an indication of the gross biochemical changes occurring in the testis of the rat during the maturation process.

CHAPTER II

REVIEW OF LITERATURE

The histological changes occurring during testicular maturation have been well documented in the albino rat (17, 26, 61), and man (10, 21, 30, 50), while the corresponding biochemical changes have received little attention. This review is the result of an attempt to obtain an understanding of the postnatal developmental morphology of the scrotal and artificially cryptorchid testis. Also, information is presented on the histochemistry and metabolism of testicular tissue.

Postnatal Developmental Histology

One of the earliest reports on developmental histology was Moore's (61) description in 1939 of changes in rat testis during maturation. Copious descriptive knowledge of the postnatal develop- of the testis of other mammals has been collected since Moore's (61) early observations.

Mancini (51) in 1960 reported on the origin and development of cells in the human testis. The fetal testis possesses two cellular types: one is the primitive spermatogonia and the other is the supporting or indifferent cell. Mancini (51) made no mention of the presence of Leydig cells in the fetal testis but Segal, et al. (69) in 1959 reported that this cell type was present in the fetus and hypertrophied during the eighth month of fetal life. Shortly after birth these cells appeared to regress and were not identifiable again until the 12th or 13th year of life. This concept of separate fetal and pubertal generations of Leydig cells has been refuted recently by Baillie (3) based on studies of the mouse testis. He felt that a misconception arose because of inadequate histological methods for demonstrating the relatively slowly growing Leydig tissues in a rapidly expanding prepubertal

testis. His observations indicated the presence of Leydig cells throughout postnatal development in the mouse testis.

The previously mentioned cellular types proposed by Mancini (51) differentiate independently during infancy. The primitive spermatogonia give rise to the germinal epithelium cells while the supporting cells become Sertoli cells. Charny (10) in 1952 reported that from birth to 4 years small seminiferous tubules lined with a single layer of ovoid cells are evident in the human testis. Between 5 and 7 years of age the tubules are still small but lumen formation can be seen in nearly all of them. No Leydig cells are present, but fibrous stroma appear in abundance. The stroma become sparse between the ages of 8 and 9 and the seminiferous tubules increase slightly in size. During the next year the tubules grow in size and become markedly coiled. They are lined by several layers of cells, many of which contain swollen cytoplasm. Leydig cells appear in limited numbers and spermatogonia are identifiable. During the 11th year the secondary spermatocytes are seen. There is also the rare appearance of a spermatid.

Sniffen (71) in 1952 and de la Balze (21) in 1960 reported on the pubertal maturation of the human testis. They observed the following: (1) during the pubertal years (11-15) seminiferous tubules are large; (2) there is active spermatogenesis up to and including the production of a few spermatozoa; and (3) Leydig cells are abundant during this time period. The mature years follow and during this period, spermatozoa are actively produced.

The histological development of the rat testis has been found to compare favorably with human development except on a different time scale. Clegg (11) in 1960 found that full spermatogenesis is established in 90-100 percent of the seminiferous tubules in the albino rat by 50 days of age. The first sperm to enter the vas deferens were noticed 13 or 14 days later, making the initial age of fertility 63-64 days.

Besides being linked closely with cellular development, spermatogenesis has also been associated with the appearance of the male hormone testosterone and the functional development of the male accessory glands. The appearance of fructose in the secretory fluids of the accessory glands was found by Davis and Mann (20) to preceed

the onset of active spermatogenesis in rabbits and bulls. It appears that the accessory organs accumulate fructose so that when the motile sperm make their appearance in the generative tract the fructose reserve is available to be utilized as a nutrient material.

Development of Spermatogenic Cycle: Albert (2) has stated that one of the most important advances in the knowledge of the testis in the last 25 years was the clarification of the spermatogenic cycle in the germinal epithelium. The cycle in the seminiferous epithelium has been reported by Leblond and Clermont (45) to be the sequence of events by which a complete series of cellular associations follow one another in time, in any given area of seminiferous epithelium.

In the rat, Leblond and Clermont (45) subdivided the cycle into 19 stages, each one of them being identified by a given step in the development of the spermatids. The 19 stages were divided into the following 4 phases:

(1) The first facet is the Golgi phase, which includes three stages and results in the fusion of proacrosomic granules into one large granule.

(2) The second state is the cap phase, consisting of stages 4-7. This facet begins with the acrosome granule flattening on the nucleus and ends with maximum development of the acrosomic cap.

(3) Phase 3 is the acrosome phase and includes stages 7-14. The aspect of the cycle ends with the head cap being loose over the nucleus, the cytoplasm condensing, and the spermatid beginning to look like a mature spermatozoon.

(4) The fourth and last state is the maturation phase, consisting of stages 15-19. Near the end of this facet the mature spermatozoon is released into the lumen of the seminiferous tubule.

Renewal of Spermatogonia: One major question posed by such workers as Moore (61) was how the spermatogonia were renewed. It was postulated that they were renewed from the Sertoli cells, but this hypothesis was discarded in 1953 when Clermont and Leblond (16) proposed a new theory for the renewal of stem cells. This original theory has been modified by Clermont (15) in 1962. The new

"Stem Cell Renewal" model, while still considered theoretical, but statistically feasible, gives an accurate representation of the maintenance of the stock of spermatogonial stem cells and of the periodic formation of generations of spermatocytes in the rat.

According to the 1962 theory (15) a pair of type A stem cells starts proliferating at stage 9 of the cycle proposed by Leblond and Clermont (45). Thereafter, the daughter cells divide successively at stages 12 and 14-15 of the cycle, their number increasing in geometrical progression. Following the third spermatogonial mitosis, a pair of cells become dormant and form new type A stem cells, which will later provide for new generations of spermatogonia. The other spermatogonial elements, arising from the third spermatogonial division differentiate into intermediate type spermatogonia, which give rise to type B spermatogonia during stage 4 of the cycle. The type B cells divide at stage 6 to produce a generation of primary spermatocytes.

Development of Interstitial Tissue: The development of interstitial tissue has been understood for somewhat longer than that of the germinal epithelium and is best documented in man and bull. Hooker in 1944 (36) and 1948 (35) described the maturation of the interstitial tissue in the bull. In the one-month-old bull, seminiferous tubules were found to be small, devoid of lumina, and sparsely distributed. Mesenchymal cells were the only cell type observed in the intertubular space. Up to 2 years, as age increased, the intertubular spaces decreased in size and the mesenchymal cells were converted to Leydig cells. After 2 years of age the Leydig cells became extensively vacuolated and increased in number and size. From 5-15 years, increased loss of vacuolization and dimunition in size of the Leydig cells were observed. Degeneration of the Leydig cells supravened after 15 years of age.

Hooker (36) reported that the life history of the Leydig cell in man and mouse was closely related to that observed in the bull. Mancini, et al. (52) recognized 2 types of Leydig cells in the adult human testis: immature and mature. The mature cells were found to contain androgens and Sniffen (71) deduced that these cells actually

produce the androgens of the testis. Wislocki (75) in 1949 and Hooker (35) in 1948 presented evidence which supports the conclusion that the Leydig cells produce androgen.

Architecture of the Mature Testis: Now that the histological components of the testis during maturation have been examined in some detail, it is important to understand how these components work together to initiate and maintain testicular function. This can best be accomplished by looking at the overall architecture of the testis and its suitability for the process of spermatogenesis.

Clermont (14) in 1958 reported that the rat testis is composed primarily of seminiferous tubules. The large surface area presented by the epithelium of these tubules makes the testis suitable for the spermatogenic process. The lumina of the tubules form the pathway for the transport of the sperm cells to the ductuli efferentes. This phenomenon of spermatozoon transport is passive, hence a fluid medium is needed. Davis and Mann (20) found this fluid to be a liquid which can be transferred along the length of the tubules. Albert (2) reported that the origin of such a fluid is not known.

The interstitial tissue is composed of membrane wedges. These wedges contain Leydig cells and are reported by Albert (2) to be bounded by 3 seminiferous tubules. Blood vessels, derived from the testicular artery, feed the wedges of connective tissue. These capillaries are in close proximity to the Leydig cells, and are arranged so the blood flows by the generative portion of the testis after contact with the Leydig cells. This organization would enable the androgens secreted by the Leydig cells to exert their influence on the germinative epithelium.

Descent of the Testis and Artificial Cryptorchidism

Descent of the Testis: The developmental histology of the postnatal mammalian testis has now been studied in detail. It is a well-known fact that in mammals the testis descends from an abdominal position to the scrotum. This phenomenon occurs at various times during the testicular development of different mammalian species (2).

In man, horse, and bull, testicular descent occurs prenatally, while postnatal descent is evidenced in the opossum. In rabbits and rodents, this event takes place during puberty. In seasonal breeders like the ground squirrel and the cotton rat, the testes alternate between scrotum and abdomen with the breeding and nonbreeding seasons, respectively. The possible mechanisms of testicular descent and the effect of abdominal retention on the structure and function of the testis will now be discussed.

The mechanism of testicular descent is not clearly understood. The gubernaculum seems to act as a guide for the descending testis, but Wells (73) in 1943 showed that excision of the gubernaculum does not prevent descent. Martins (53) in 1943 found that substitute testes in the form of paraffin pellets can be made to descend in castrate rats by the administration of testosterone. Because of this fact, he concluded that descent is determined by androgens affecting the testis and accessory structures. It is postulated that the human fetal testis produces androgens responsible for descent. This supposition has arisen from the fact that Segal (69) has shown Leydig cells to be present in the prenatal testis and histochemical studies by Mancini (52) indicated that Leydig cells contain sterodial material.

Artificial Cryptorchidism: The retention of the testis in the abdominal cavity is termed cryptorchidism and can be experimentally produced in laboratory animals by translocating a testis into the abdominal cavity. The effects of cryptorchidism on the histological appearance of the human testis have been the subject of many studies. However, few new observations have been denoted since the early study by Cooper (19) in 1929. Cooper studied abdominal and scrotal testes in man at various ages ranging from birth to senility. His observations led Cooper to the following conclusions: "(1) the farther the prepubertal testis descends, the more similar it is to its scrotal mate; (2) sperm cells are rare in the retained testes, but occassionally may be found in testes held at the external ring; (3) the Leydig cells are not affected adversely by retention, nor are they more numerous." These observations in man have been confirmed in general by Nelson (64) in the albino rat.

Nelson (64) in 1951 observed that prolonged residence of the testis within the abdominal cavity in the rat led to progressive loss of all spermatogenic cells, including spermatogonia. The ability of the testis to renew spermatogenic activity was directly related to the number of tubules with spermatogonia present. In these rats the loss of spermatogonia did not occur until the testis was retained for more than a month, and the number of tubules without spermatogonia did not reach 50 percent until four months later. Irreparable damage to all tubules appeared after seven months.

The damage to the germinal epithelium is much more noticeable and at an earlier time period than the damage to the spermatogonia. Moore (62, 63) in 1922 and 1923 reported on the damage to the germinal epithelium in the rat, produced by experimental cryptorchidism. Degeneration was noticeable after 7 days and the germinal epithelium was completely gone after 21 days. As the germinal epithelium degenerated the cells united into discrete syncytial masses, similar to giant cells, in the epithelial layer, or free in the lumen of the seminiferous tubules. Clegg (13) in 1963 studied the changes in the Sertoli cells in the rat testis during artificial cryptorchidism. His findings indicated that the Sertoli cell nuclei increase significantly in number between the 10th and 21st postoperative day. This increase in nuclei is accompanied by a decrease in nuclear size; it is considered that these changes may be due to amitotic division. The Sertoli cells appear to be phagocytic and are able to ingest particles directly introduced, such as India ink. It was, therefore, postulated that these cells are capable of ingesting degenerated spermatogonia and spermatocytes.

Clegg (12) in 1961 studied the quantitative changes in the Leydig cells of the rat during cryptorchidism. Artificial cryptorchidism was followed on the 21st day by a transient increase in the number of Leydig cells. At other times, the total number of these cells are not significantly altered, but the proportion of non-senile Leydig cells is increased over the whole experimental period. The transient rise in cell number is considered to be due to the operation of the normal pituitary-gonad relationship and the subsequent fall in cell numbers to diminished ability of damaged interstitial tissue to react to gonadotropins.

Because of the above findings, the effect of cryptorchidism on the ability of the testis to synthesize testicular androgens has been studied. Llaurado and Dominguez (46) in 1963 divided immature male rats into 3 groups: (1) control, (2) hypophysectomized, and (3) unilaterally cryptorchid. Three weeks after the operations, the ability of the cryptorchid testis to convert progesterone to Δ^4-androstene-3,17-dione and testosterone was compared with its scrotal mate. In all cases, the conversion of the scrotal testis was much greater than the cryptorchid as was the testis from the hypophysectomized animals to which had been administered Human Chorionic Gonadotropin (HCG). Apparently, there is a limit to the response of the cryptorchid testis which is much lower than that of the eutopic organ.

It has been shown that cryptorchidism results in serious damage to testes of some mammals. Birds, however, maintain abdominal testes throughout life and are able to carry on active spermatogenesis. In seasonal breeding mammals, the testes reside in the scrotum only during the breeding season. Ewing, Green, and Stebler (24) reported that in the seasonal breeding cotton rat (Sigmodon hispidus) the testes become greatly atrophied during their residence in the abdominal cavity. At the onset of the breeding season, however, the testes begin to grow in size while still in the abdominal cavity. Upon testicular descent, these glands continue to develop, and are assumed to resume active spermatogenesis with no apparent damage due to the previous abdominal period. These facts concerning the abdominal position of the testis in birds and seasonal breeding mammals have led to studies to determine the causes of testicular degeneration during cryptorchidism in animals with scrotal testes.

Moore (62) in 1922 eliminated such factors as infection, greater pressure applied to the testis, severance of blood or nerve supply, and scrotal sac connections as being causes of testicular degeneration during cryptorchidism. The factor proposed as the cause was the differential temperature between scrotum and abdomen. Albert (2) reported that scrotal temperature is 1-8° C. lower than the corresponding body temperature of all animals investigated to date. Moore confirmed the above theory when he wrapped the testes of rams with wool batting and found that these animals sterilized themselves with their own body

heat. Hotchkiss (38) in 1944 observed that application of water 5° C. above normal body temperature to the testis of guinea pigs caused temporary sterility. He also reported on the deleterious effects of increased temperature in man. Fever, diathermy, or heating of the human testis by other methods produced temporary depression of sperm count.

Fukui (27) in 1923 approached the concept of the thermoregulatory function of the testis from another standpoint. A cooling apparatus employed on the exterior, proximal to inguinal and abdominal testis experimentally retained was effective in reducing the temperature. A testis treated in such a manner could be maintained in a nearly normal condition, while its mate, also experimentally retained but not cooled, showed marked degeneration.

The reason for the effect of temperature on reproduction in the male is not clearly understood, but it is generally accepted that spermatogenesis in mammals can proceed only at an optimum temperature (41). Engberg (23) noted a severe androgen deficiency in bilaterally cryptorchid men. This work and the same findings in a variety of animals (33, 39) have led to the belief that metabolism of testicular hormone is altered by increased temperature, thus causing testicular atrophy and the cessation of spermatogenesis. In general, then, the cryptorchid testis is capable of producing androgens, but, depending on species, in lower amounts than control animals. The longer the state of cryptorchidism exists, the more deficient is the capacity to secrete.

Chemical Composition and Metabolism of Testicular Tissue

Histochemistry: The morphological and histological structure of the scrotal and cryptorchid testis has been examined in some detail. The occurrence of chemical substances present in the various cellular structures of the testis will now be explored.

Much of the work concerned with the chemical composition of the testis has been of a histochemical nature. The methods used for staining the various components of tissues are numerous. A good review of some widely used histochemical procedures was prepared by Elftman (22) in 1952.

No histochemical studies could be found concerning the protein content of the testis. Horn (37) in a nutritional study showed that protein in the diet was necessary for testicular maturation. Immature albino rats 22 days of age were observed to lose weight when fed a protein-free diet, and after 30 days, survival was markedly curtailed. The omission of dietary protein prevented testicular maturation and histochemical techniques revealed an increase in tubular lipids. Permanent testicular damage was not apparent in rats returned to a regular 18 percent casein diet after 30 days on the protein-free ration. Testis maturation was not related to body weight recovery and the testis of the experimental rats had recovered to control values after 30 days refeeding on the protein diet. Accessory sex organ weight on the other hand, were only partially recovered after 30 days refeeding on the 18 percent casein ration, which indicated to Horn that spermatogenic maturation and androgen release were not operating on the same level.

Montagna and associates published a series of 3 papers on the distribution of lipids and carbohydrates in the human testis. In a 1951 article Montagna and Hamilton (60) studied lipid distribution in the human testis using histochemical methods. Lipids were observed in Sertoli cells and the fibroblast-like cells of the interstitium. Leydig cells were found to vary greatly as to lipid content, some containing large amounts and others none at all. Lipid was also observed to be present in the epithelium of the ductuli efferentes, but was not found in the ducti epididymides. This paper was followed by two others by Montagna (58, 59) in 1952 dealing with the histochemical distribution of glycogen and other carbohydrates in the human testis. Glycogen was found to be present in the spermatogonia, small and growing primary spermatocytes, and also in Sertoli cells. There was, however, no distinguishable glycogen in mature primary and secondary spermatocytes. Non-glycogen carbohydrates were found in the Leydig cells. Mancini and associates (52) in 1952 confirmed Montagna's findings and also observed the presence of two types of Leydig cells: (1) immature, containing lipids, but not sterodial material, and (2) mature cells which contained steroids.

Long and Engle (47) in 1952 performed a cytochemical study on

testicular biopsy tissue obtained from 9 human sterility patients. The Sertoli cell lipid was that of an age group approximately three years older than controls. Quantities of cholesterol were present in the Sertoli lipid. A cholesterol determination of interstitial cells proved negative and was interpreted to be indicative of a rapid conversion of cholesterol into hormone. The increased amounts of Sertoli cell lipid were believed to be an indication of decline in cellular activity. The increase in lipid content was found to be accompanied by a decrease in glycogen.

Cavazos and Melampy (9) in 1954 confirmed Montagna's (59) observations in a comparative study of carbohydrates in the adult vertebrate testis. Glycogen was found in the Sertoli cells, while non-glycogen carbohydrate was observed in the interstitial cells, the basement membrane of the seminiferous tubules, and the germ cells. Another paper published in the same year by Melampy and Cavazos (57) dealing with lipids in the adult vertebrate testis confirmed the earlier findings of McEnery and Nelson (55). Phospholipids were found in the Leydig cells and the Sertoli cells of all mammals used in the experiment.

Leblond (44) in 1950 studied the distribution of carbohydrates in the testis of the adult rat. Periodic-acid reactive carbohydrates were found in Leydig cells, seminiferous tubules, and the acrosome of spermatozoa. Lynch and Scott (49) in 1951 studied the lipid distribution in adult rat testis and found lipid present in Sertoli and Leydig cells. They concluded that Sertoli cells have a nutritive function because in absence of spermatogenesis, whatever the cause, lipid accumulates in the Sertoli cells adjacent to the basement membrane of the seminiferous tubules. Unilaterally cryptorchid rats were also studied by Lynch and Scott (49). In these animals, a loss of germinal epithelium was observed, accompanied by an accumulation of lipid in the Sertoli cells. Leydig cells containing lipid remained unchanged, while hyperplasia was observed in Leydig cells which contained no lipid.

The histochemistry of Leydig tissue lipids has recently been studied by Niemi and Ikonen (65) in rats from the 3rd to the 50th postnatal day. Prepubertal Leydig cells were found to contain sudanophi-

lic lipid material, whereas those of the mature animal did not exhibit any stainable lipids. On the basis of these results and those found by Mancini (52) in 1952 and Roosen-Runge (68) in 1959, Niemi and Ikonen concluded that there are two different generations of Leydig cells in the rat. Baillie (3) reported that there are not two generations of Leydig cells in the mouse testis, and questions the presence of separate fetal and pubertal generations in other species.

Wislocki (75) in 1949 reported on the seasonal changes in testicular composition of the deer. No glycogen was found in the Leydig cells in the mature male deer. Glycogen was found to be present in the Sertoli cells of the active testis, while in the inactive testis, no glycogen was present in the Sertoli cells but was observed in many of the tubular lumina. Lipid was observed in the interstitial cells throughout the year, but was present in higher concentrations during the active season. Lipid was also present in the "kinoplasmic droplet" (29) (a cytoplasmic residuum of unknown function) which is lost by the spermatids during maturation into mature germ cells.

Quantitative Chemistry: Although much of the work dealing with the chemical components of the testis has been histochemical or qualitative in nature, there are some quantitative studies recorded in the literature. These dealt primarily with lipid components, such as phospholipids and fatty acids.

Cole (18) in 1956 reported on the highly unsaturated fatty acids (HUFA) in embryonic and adult testis tissue of the golden hamster. His results indicate that the hamster testis reaches a peak in oxidation potential at 55-65 days of age. Kirschman and Coniglio (40) in 1961 worked with polyunsaturated fatty acids in tissue of growing rats. Concentrations of polyunsaturated fatty acids were determined in rats of weanling, 3 months, and 6 months of age. The concentrations of these acids were found to change with age, the most marked change being a large increase in pentaene levels of testicular tissue between the ages of 3 weeks and 3 months.

McEnery and Nelson (56) in 1953 estimated the concentrations of various organic phosphorus compounds (pentose nucleic acid phosphorous, desoxypentose nucleic acid phosphorous, total nucleic

acid phosphorous, acid soluable phosphorous, and lipid phosphorous) in the testis of the rat between 10 and 180 days of age. The concentration of all the phosphorous containing fractions, except lipid phosphorous, decreased with increasing age when expressed as mg. phosphorous per 100 gm. testis. However, in terms of absolute values (amount per pair of testes) each of the phosphorous fractions showed a progressive increase with age. The authors concluded "that the amounts of several phosphorous fractions which could be detected at various ages depends on the types of cells and on the number of cells which are present at those ages except in the very young testis where an additional relationship exists between the rapid rate of testicular growth and the concentration of pentose nucleic acid and soluable phosphorous." Gottfried and Rapport (28) in 1963 determined the concentration of a particular phospholipid type (plasmalogens) in heart, lung, liver, spleen, and skeletal muscle of rats as a function of age. There was found to be no consistant change in plasmalogen concentration of all tissues analyzed between 1 and 8 weeks of age.

Wolf and Leathem (77) in a nutritional study observed that the immature rat testis contained 85 percent water, 10.5 percent protein, 4.5 percent lipid, and detectable glycogen. This composition was maintained for 20 days in rats fed on a 20 percent casein diet. Diets of lower nutritive value decreased the testis protein and decreased testicular growth, but did not alter the lipid or glycogen content. A 5 percent casein diet was found to reduce testis protein and increase glycogen concentration. In both cases of low protein diets, the water content of the tissue was increased. Addis and colleagues (1) in 1936 determined the effects of fasting on the protein concentration in various tissues of the adult male rat. The tissues examined were muscle, skin, skeleton, liver, alimentary tract, pancreas, kidney, spleen, heart, testis, adrenals, and eyes. A 7 day fast was found to have no effect on the protein content of testis, adrenals, and eyes, but resulted in protein loss in all other tissues analyzed.

The effect of early and late weaning on spermiogenesis was determined in the immature male rat by Kubat and workers (42) in 1960. The animals were fed on high fat (HF), high glycid (HG), or high protein (HP) diets. No differences in weight gains were found in

rats fed HF or HG diets, but the HP animals were always smaller. Spermiogenesis was impaired in rats weaned prematurely and fed on HG or HP diets, but an HF diet seemed to prevent this damage to the testis. The authors conclude that the period between 18 and 30 days is of particular importance in the development of the rat and that the quality of food supplied during that time period may be of decisive importance in later life.

Metabolism: The metabolism of testicular tissue has not been extensively studied. Tepperman, Tepperman, and Dick (72) in 1949 investigated the metabolism of rat testis in vitro. The animals were rendered unilaterally cryptorchid and the abdominal testis was compared to its scrotal mate. Atrophy of the seminiferous tubules was found in the cryptorchid testis. The endogenous oxygen uptake of the scrotal mates was found to be lower than those of testis slices of the treated animals. It was found that aerobic glycolysis of a cryptorchid testis does not differ from that of a scrotal gland. Androgen added in vitro produced a lowering of the endogenous oxygen uptake in the intact testis. This effect is magnified in the cryptorchid gland.

Paul and workers (66) in 1953 studied carbohydrate metabolism in the testis of rats fed nitrofurans. The endogenous metabolism of the testis from Furacin-fed rats was higher than that of control testis and added glucose did not cause an increased oxygen uptake. This response of the testis from treated animals resembles that shown by other workers for the cryptorchid testis. A loss and regeneration of certain germinal elements was coupled with a corresponding disappearance and return of citrate synthesis. The ability of the treated testis to utilize glucose returned to control levels when tissue recovery had progressed nearly to completion as evidenced by the presence of mature spermatozoa.

Madhu and Macleod (50) in 1961 studied the metabolism of testicular tissue in mature and immature rats. They used animals ranging in age from 18 days to maturity. Endogenous oxidation of testis tissue of control animals decreased from 39-50 days of age. Little or no oxidation of glucose was evidenced until the 25th day of age, and beyond that time there occurred an incremental increase until maturity. Fruc-

tose was not efficient as a substrate in the immature testis until the appearance of fully mature spermatids and spermatozoa. At this same time, lactic acid production from fructose became apparent. Upon stimulation by Human Chorionic Gonadotropin (HCG) or Follicle Stimulating Hormone (FSH), the immature rat testis was found to utilize fructose as a substrate to a much greater extent than did the testis of the same age control animals. Blackshaw (6) in 1962 studied the utilization of glucose and fructose by adult mouse testis in phosphate-containing media. It was observed that inorganic phosphate increased the accumulation of lactic acid from glucose and in the absence of exogenous substrate, lactic acid production was severely depressed. The same type of response to inorganic phosphate was observed with fructose, but to much less extent.

A recent paper on the testicular metabolism of fatty acids was published by Hall, Nishizawa, and Eik-Nes (32) in 1963. These workers isolated several fatty acids from rabbit testis and found evidence for the de novo synthesis of palmitic and stearic acids from C^{14}-labeled acetate. Administration of Interstitial Cell Stimulating Hormone (ICSH) did not produce demonstrable stimulation of the synthesis of the fatty acids in vitro, although the hormone stimulated the production of testosterone-C^{14} by the same tissue. It was concluded from these findings that fatty acid biosynthesis is probably not influenced by the mechanisms by which tropic hormones increase steroid function.

The studies reviewed indicate that histological changes occurring in the testis during maturation are well documented. The histochemical changes have also been investigated in detail. On the other hand, very little information seems to be available on the biochemical changes which take place during testicular maturation. These biochemical changes could very possibly control the cell division which occurs during spermatogenesis. Therefore, there is an evident need for a study on the biochemical events which occur during maturation of the testis.

CHAPTER III

MATERIALS AND METHODS

Experimental Design and Pretreatment

One hundred twenty-eight 14-day-old male albino rats of the Holtzman strain were used in this experiment. The animals were equally divided into 2 groups, and 1 group was rendered bilaterally cryptorchid. The resulting 2 groups of 64 animals were each divided into 8 subgroups containing 8 animals per subgroup. Beginning on the 14th day of age, 1 subgroup of control rats and 1 subgroup of cryptorchid animals were sacrificed weekly for a period of 8 weeks. Because of the fatality rate within subgroups, the number of animals sacrificed per week varied. The number of control animals sacrificed weekly from week 1 (14th day of age) to week 8 (63rd day of age) was 8, 8, 7, 5, 7, 7, 7, and 7, respectively.

In the cryptorchid group some animals died, but this was secondary to another problem encountered. This problem resulted from the fact that in some of the rats the testis descended back into the scrotum, making it necessary to eliminate these animals from the experiment. Testicular descent increased with age and by the 7th experimental week (56th day of age) only 2 animals remained bilaterally cryptorchid. These 2 rats were saved and used on the terminal week of the experiment, leaving a void in the cryptorchid data at 56 days. Therefore, the number of cryptorchid animals used per week during the 8 week experimental period was 8, 8, 7, 5, 5, 4, 0, and 2, respectively.

Each week during the experiment, testes from rats were removed, stripped of fat, weighed on a Roller-Smith torsion balance, and then pooled according to subgroup. The pooled tissue was minced with scissors and 600 mg. removed to be analyzed for total carbohy-

hydrates and total protein. Lipid was extracted from the remainder of the minced tissue. The lipid-free residue was dried, weighed, and assayed for glycogen and Kjeldahl nitrogen.

Lipid Analysis

Lipid Extraction: Lipids were extracted from the testicular tissue by the following modification of Gray's (31) method. The tissue was minced with 5 volumes (w./v.) of chloroform:methanol (2:1, v./v.) and allowed to stand overnight at 5° C. Extracts were filtered on Buchner funnels and the residues were washed twice by resuspending in 1 volume of chloroform:methanol (2:1). The extract and washings were combined, transferred to a separatory funnel, and washed with 1 volume of tap water by gently stirring the mixture with a mechanical stirrer. After 2 hours, the chloroform layer was removed and washed twice with 0.5 volumes of 0.5 percent KCl solution. The washings were discarded and the chloroform solution kept at -20° C. overnight to freeze out the remaining water, then filtered in the cold, and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo at 40° C. The lipid was then redissolved in chloroform and diluted to 10 ml. total volume. This solution was centrifuged to remove any Na_2SO_4 particles present. The clear to yellow supernate was removed and a 1.0 ml. aliquot plated into a tared planchet and dried overnight in a dessicator. The planchet was then weighed at 1 hour intervals until a constant weight was established, thus determining total lipid content gravimetrically.

Separation of Phospholipid from Neutral Lipid: The total lipid was separated into neutral fats and phospholipids by silicic acid chromatography following the method of Borgstrom (7). A 5 gram column of silicic acid was used in all separations. For separation the total lipid sample was concentrated to 1 ml. under a stream of nitrogen gas and quantitatively transferred onto the column. The neutral fats were then eluted by percolating 100 ml. of absolute chloroform through the column under a positive pressure of nitrogen sufficient to provide a flow rate of 1 ml. per minute. The phospho-

lipids, which were quantitatively absorbed on the column, were then eluted by first forcing 75 ml. of chloroform:methanol (4:1, v./v.) through the column with nitrogen and then 75 ml. of methanol. The solvent was removed in vacuo at 40° C. from the neutral lipids and the phospholipids. Each residue was redissolved in chloroform to a final volume of 10 ml. and neutral phospholipid concentrations were determined gravimetrically by the method described previously for total lipid. In all cases the percent recovery, based on amount of total lipid put on the column was 100 ± 3.

Saponification of Neutral Lipids: Saponification of the neutral lipids into fatty acids and unsaponifiable lipids was accomplished using a modification of the technique described by Fairbairn (25). The organic solvent was evaporated from a 5 ml. aliquot of the neutral lipid fraction with a stream of nitrogen gas. The lipid residue was dissolved in ether and washed twice with 5 ml. portions of 0.5 percent Na_2CO_3 solution to remove the non-esterified fatty acids. The aqueous solutions were discarded, ether evaporated, and lipid residue suspended in normal methanolic NaOH. Saponification of the glycerides was accomplished by refluxing this suspension under a stream of nitrogen for 2 hours on a steam bath. At the end of this period the reflux condenser was removed and approximately half of the methanol was allowed to evaporate. Ten volumes (50 ml.) of ether were added to the residue, the mixture was transferred quantitatively to a separatory funnel, and washed 5 times with 15 ml. portions of glass distilled water.

Phospholipid Analysis: The inorganic phosphate content of the total lipid and phospholipid fractions were determined using Böttechers (8) modification of the original proceedure described by Bartlett (4). The phospholipids were analyzed for plasmologen content using the method of Rapport and Alonzo (67), and Kjeldahl nitrogen content of the phospholipids was determined by the method of Lang (43).

Other Chemical Analyses

Preparation of Lipid Free Extract: The residue from the total lipid extraction was dried and weighed. It was then digested with 3 volumes of 30 percent KOH for 20 minutes in a boiling water bath. The resulting solution was cooled and brought to a final volume of 5 ml. with glass distilled water. An aliquot of lipid free extract was then analyzed for Kjeldahl nitrogen.

Glycogen: Glycogen content of the lipid free solution was determined using a modification of the method of Seifter, et al. (70). A 0.2 percent anthrone solution (in concentrated H_2SO_4) was prepared. To this solution was added, with cooling, 0.5 volumes of glass distilled water. Seifter's experimental procedure was then followed per se with the exception that 15 ml. of the diluted anthrone reagent were added to each tube instead of the prescribed 10 ml. of undiluted reagent.

Total Carbohydrate: The 600 mg. of freshly minced testis tissue not used in lipid extraction was homogenized in enough ice cold 0.14 M KCl to make a 20 percent (w./v.) homogenate. A manually operated, all-glass Ten Broeck homogenizer was used to prepare the tissue. Two ml. of 30 percent KOH were added to 0.5 ml. of the 20 percent homogenate and digested in a boiling water bath for 20 minutes. After the digestion period, the solution was cooled and diluted to a final volume of 3.0 ml. with glass distilled water. Undiluted aliquots were used to assay for total carbohydrate using the modification of Seifter, et al. (70) previously described for glycogen determination.

Total Protein: One-tenth ml. of the 20 percent KCl homogenate was diluted to 10 ml. with glass distilled water. Aliquots of this dilution were analyzed for total protein using the Folin-phenol method as described by Lowry, et al. (48).

CHAPTER IV

RESULTS AND DISCUSSION

The object of this study was to provide information on levels of some biochemical constituents in the scrotal versus cryptorchid testis of the albino rat during postnatal development. This study was of a preliminary nature, and it is hoped that the findings presented here will lead to more definitive studies in the area of testicular metabolism. The biochemical criteria measured were chosen because they constitute the basic structural components of all vertebrates and because the techniques for assay were readily available. The results are presented in two main categories: scrotal and cryptorchid testis experiments.

Scrotal Testis Experiments

Body Weight and Testis Weight: The growth rate of the animals used in this experiment, along with the testis weight, is shown in Figure 1. Each point on the body weight graph represents average weight in grams of the rats used each week throughout the experiment. Testis data represents the average value of the paired testis weight. The average values in both cases are plotted versus time in days.

The rate of body growth from 14-65 days seems to continually increase. The rate of testis growth, however, is the greatest during the period between 28 and 49 days. This growth spurt was found to begin at the time of testicular descent and end at the time of testicular maturation reported by Clegg (11) in 1960. This data would indicate that the greatest testicular growth rate occurs while the testis resides in the scrotum and that growth rate diminishes after sexual maturity.

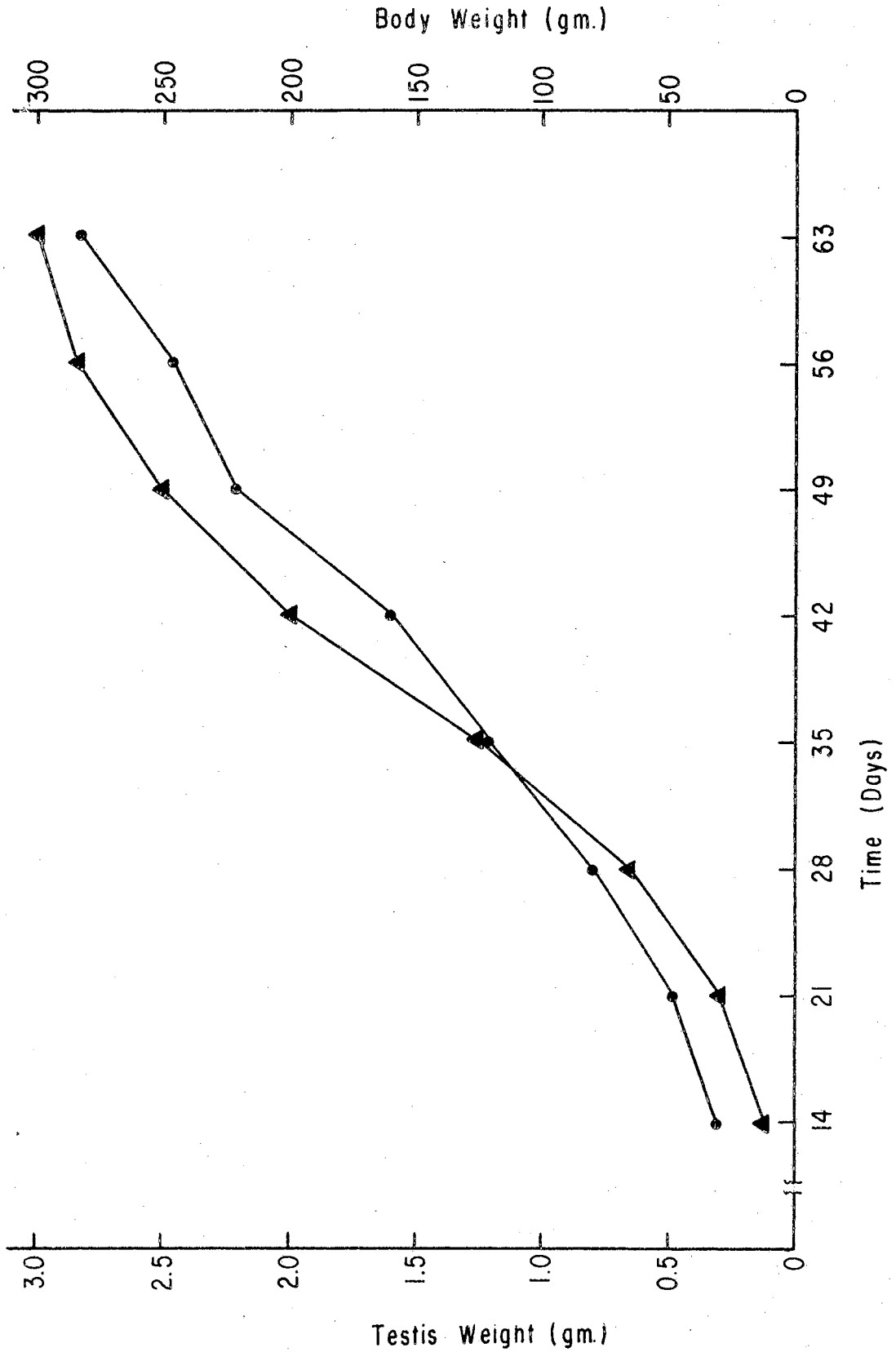

Figure 1. Changes in Body Weight and Testis Weight of Control Rats During Postnatal Maturation. ▲, testis weight; ●, body weight.

Protein Content: The total protein content seems to increase throughout the duration of the experiment whether expressed in mg. per gm. of testis or mg. per pair of testes. The increase in protein content per pair of testes is probably due to the constant increase in testis size. Figure 2 illustrates the change in protein content with respect to time. Note the apparent peak at 28 days when expressed in mg. per pair. The 28-day time period is the time of testicular descent, hence it might be postulated that a change in the protein content of the testis plays an important part in descent into the scrotum. Martins (53) has postulated that the gonadotropins serve an important part in the descent of the testis. The increase in protein content at 28 days might be explained by a transient increase in gonadotropins which serve to stimulate androgen production and protein synthesis by the testis. At first inspection, it appears that the 28-day value is out of line. However, the protein content was repeated in this laboratory on another group of 28-day old animals and found to agree closely with the initial value.

Wolf and Leathem (77) in 1955 reported that the immature rat testis contained 10.5 percent protein when expressed on a wet weight basis. The exact age of the animals was not given. Calculation from the data in Figure 2 indicates that the protein content at 35 days of age was 9 percent. The 35-day value was chosen for comparison because it is midway between the start of the experimental period and the time of maturity of the rats. This value agrees favorably with that found by Wolf and Leathem.

Carbohydrate Content: The total carbohydrate content is shown in Figure 3. There appears to be a rapid increase from 14-21 days when expressed in mg./gm. testis. The graph then shows a plateau with another increase between 56 and 63 days of age. On the other hand, the data indicates a nearly proportional increase in total carbohydrate when expressed as mg./pair of testes. This proportional change could be explained by the increase in testis size during the experimental period.

The apparent rise between 14-21 days might be explained by the observations of Madhu and Macleod (50) that little or no glucose

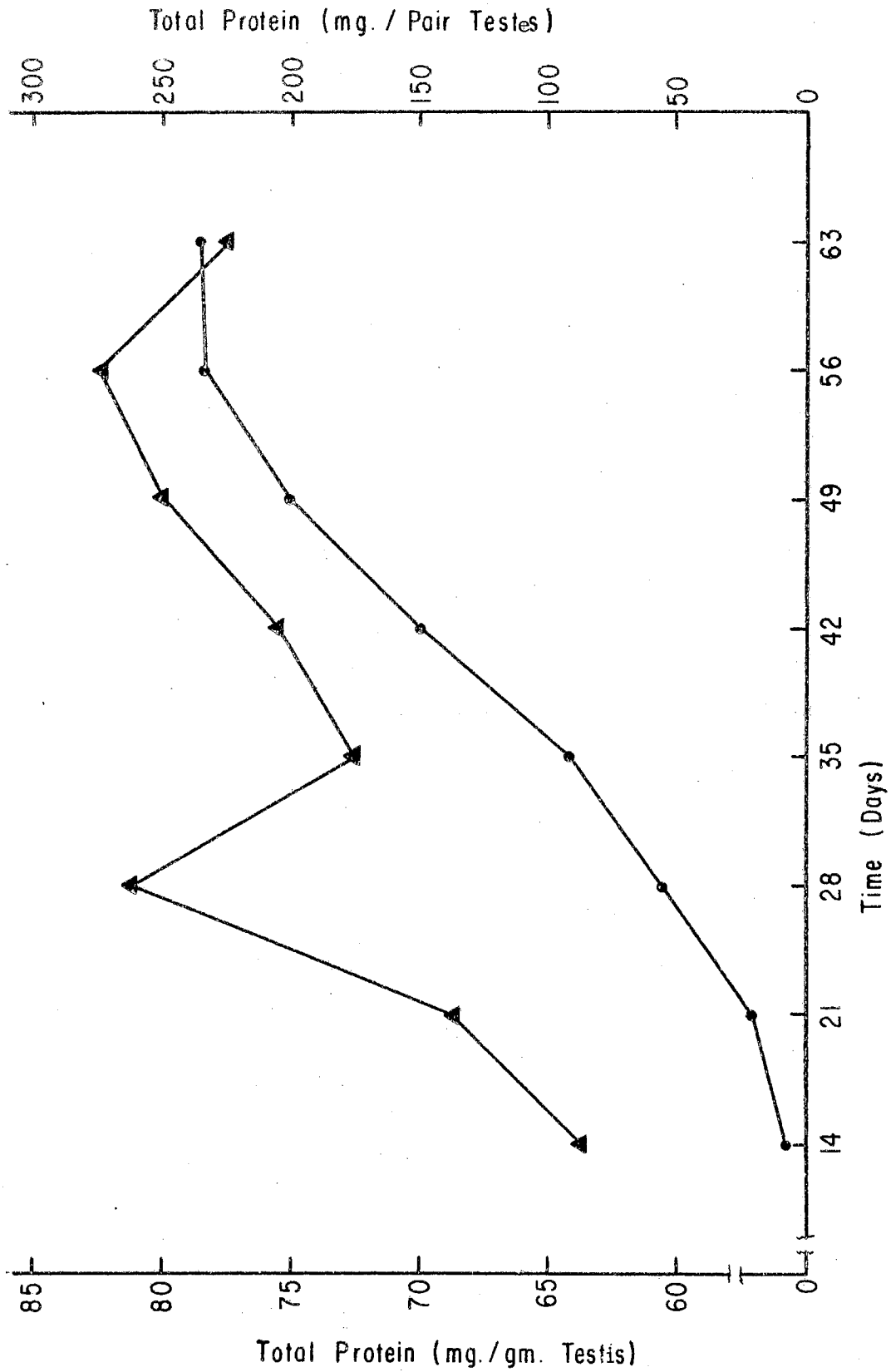

Figure 2. Changes in Testicular Protein Levels of Control Rats During Postnatal Maturation. ▲, mg./gm. testis; ●, mg./pair testes.

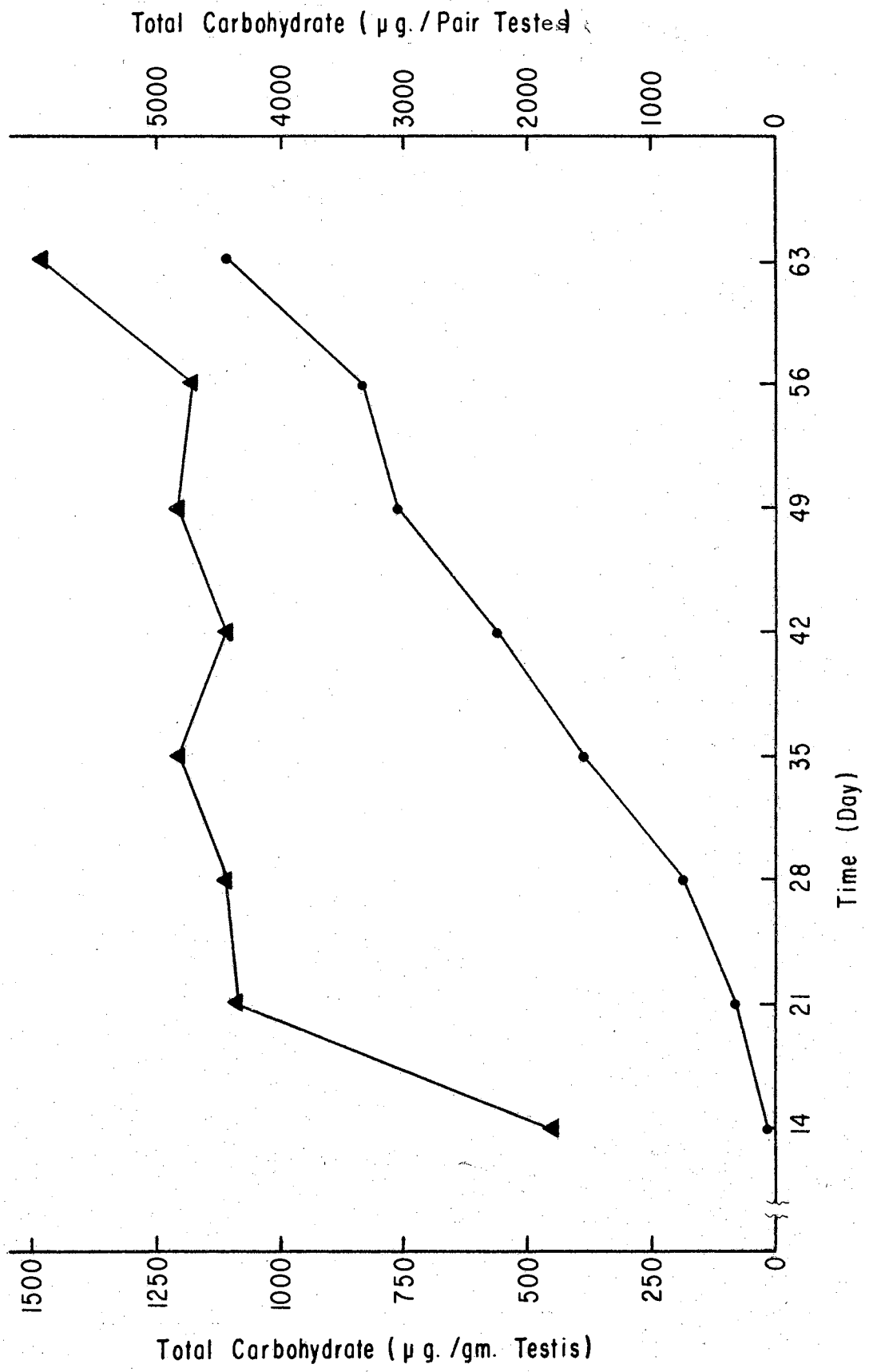

Figure 3. Changes in Testicular Total Carbohydrate Content of Control Rats During Postnatal Maturation. ▲, mg./gm. testis; ●, mg./pair testes.

was utilized by the immature rat testis until day 25. Thereafter, an incremental increase until maturity was evidenced. If this is a true observation, one might expect corresponding changes in testicular glycogen levels. Figure 4 shows the glycogen content of the testis with respect to age. The data, when expressed as mg./gm. testis are erratic, but there appears to be a decrease from 14-21 days, followed by an increase from 21-35 days. From the 35th day the glycogen content seems to decrease slightly. The decrease from 14-21 days might indicate a breakdown of glycogen resulting in a corresponding increase in glucose. The subsequent rise from 21-35 days might be explained by the increased glycogen storage of mature Sertoli cells. Clermont and Perey (17) in 1957 reported that the Sertoli cells increase in number between 21 and 35 days of age in the albino rat.

The glycogen content expressed in mg./pair of testes exhibits an increase throughout the experiment with an apparent surge between 35-49 days. The continual increase might be explained on the basis of increased testes size, but this would not account for the big spurt during the two-week period from 35-49 days. As stated previously, the Sertoli cells mature at about 33 days and the testis of the rat achieves sexual maturity at approximately the 50th day of postnatal development. Montagna and Hamilton (59), and Mancini et al. (52) have found glycogen to be present in the Sertoli cells, primary spermatocytes, and mature spermatozoa. Since all the former cell types increase in number between 35 and 50 days it would seem logical to expect an increased glycogen content during the same time period.

Wolf (76) in 1954 found the glycogen content of immature rat testis to be between 0.1 percent and 0.15 percent when based on percent wet weight. Calculations from Figure 4 show the range of glycogen content to be from a low of 0.062 percent at 21 days to a high of 0.10 percent at 35 days. These data correspond to Wolf's findings, but an exact comparison can not be made because he did not specify the age of the rats.

Lipid Content: Total lipid, as exemplified by Figure 5, shows

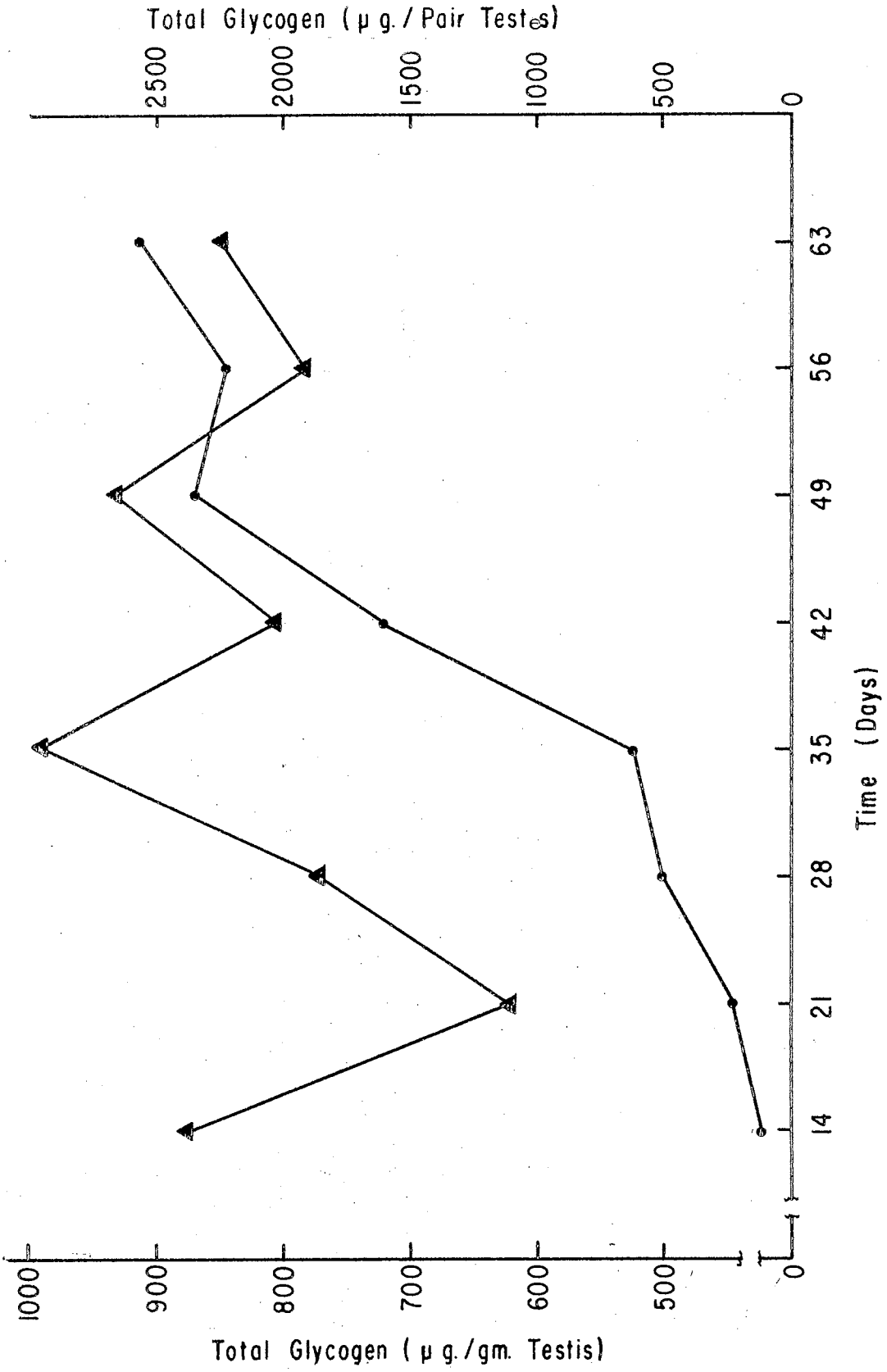

Figure 4. Changes in Testis Glycogen Levels of Control Rats During Postnatal Maturation. ▲, µg./gm. testis; ●, µg./pair testes.

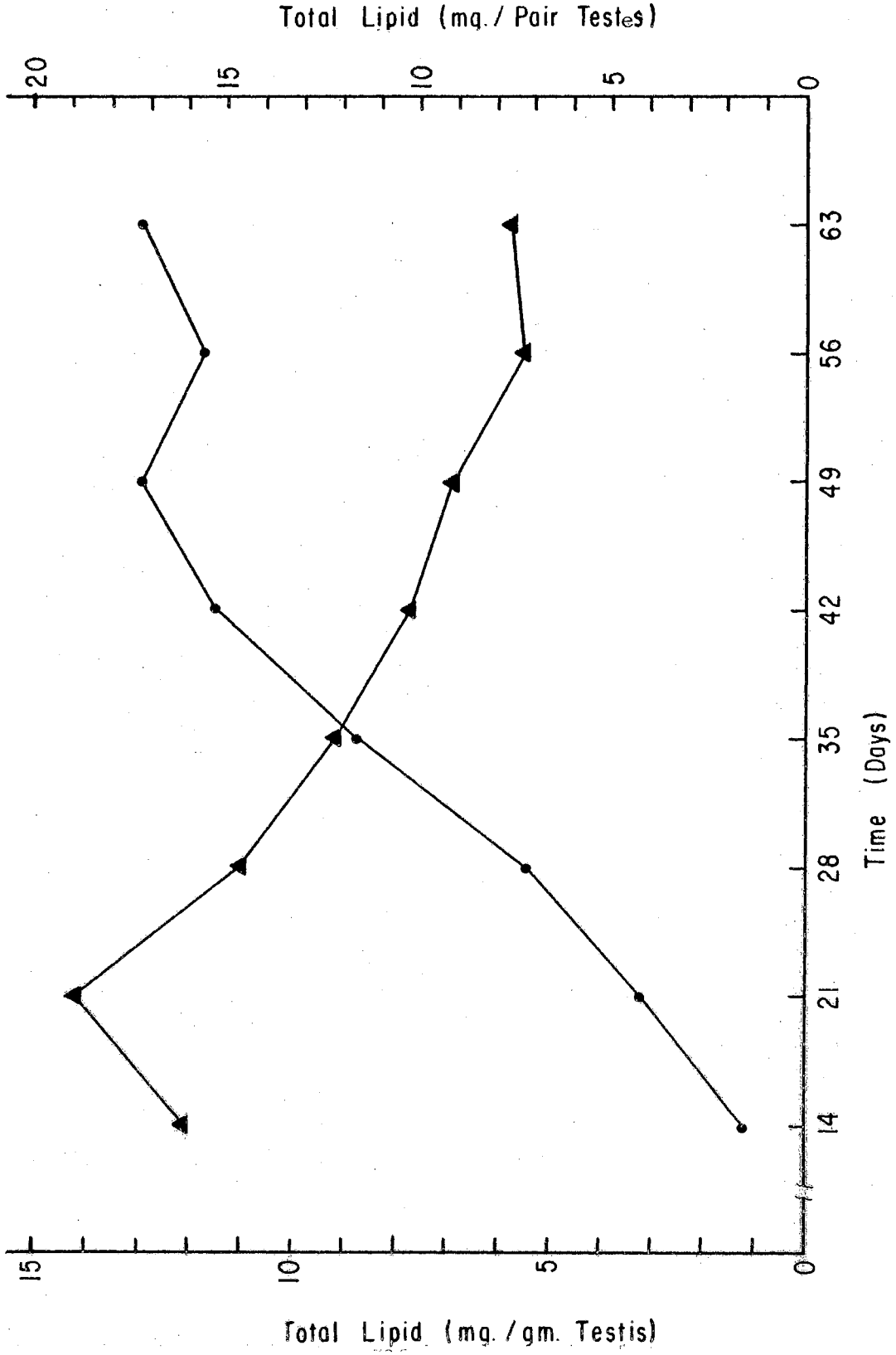

Figure 5. Total Lipid Changes in the Testis of Control Rats During Postnatal Maturation. ▲, mg./gm. testis; ●, mg./pair testes.

a steady increase per pair of testes. This is probably due to the increase in cell types and numbers resulting in an increased testis size and weight. On a mg. per gm. basis the opposite trend is apparent. After a slight elevation at 21 days total lipid content decreases continually to day 56 when the total lipid seems to stabilize. This could be because the testis is increasing in size and cell number faster than lipid is being synthesized, or that lipid is being utilized to a greater extent.

Wolf and Leathem (77) in 1955 reported that the immature rat testis contains 4.5 percent lipid, while calculation from data in Figure 5 indicates that the 35 day old rat testis is composed of 1.2 percent lipid. The discrepency in total lipid values may stem from the fact that different extraction techniques were employed. The testicular lipids found in this experiment were extracted using a modification of Gray's (31) procedure. The technique employs a chloroform:methanol (2:1, v./v.) extraction medium followed by a water and KCl wash to remove any non-lipid material. Completeness of the extraction method was tested by suspending the extracted tissue in chloroform:methanol (2:1) and refluxing for 24 hours. This second extract was then washed in exactly the same manner as the first. In the tissue so examined, 97 percent of the total lipid was in the initial extract. Wolf and Leathem (77) used a technique first described in 1905 and later modified in 1948. The lipids were extracted by first refluxing with 95 percent ethanol and then diethyl ether, but the lipid fraction was not washed. On the basis of the above technical differences, it seems entirely possible that the lipid fraction described by Wolf and Leathem contained some non-lipid material.

The total lipid was separated into neutral and phospholipid fractions. For the sake of convenience the neutral lipid and phospholipid data are presented together in Figure 6 and expressed only in mg. per gm. of testis. Other data concerning these lipid fractions are presented on page 46 of the appendix.

Figure 6 shows the phospholipid and neutral lipid data. There seems to be no particular pattern which the phospholipids follow. Williams, et al. (74) in 1945 and Biezenski, et al. (5) in 1963 reported that the phospholipid content of female rat kidney, liver, uterus, and

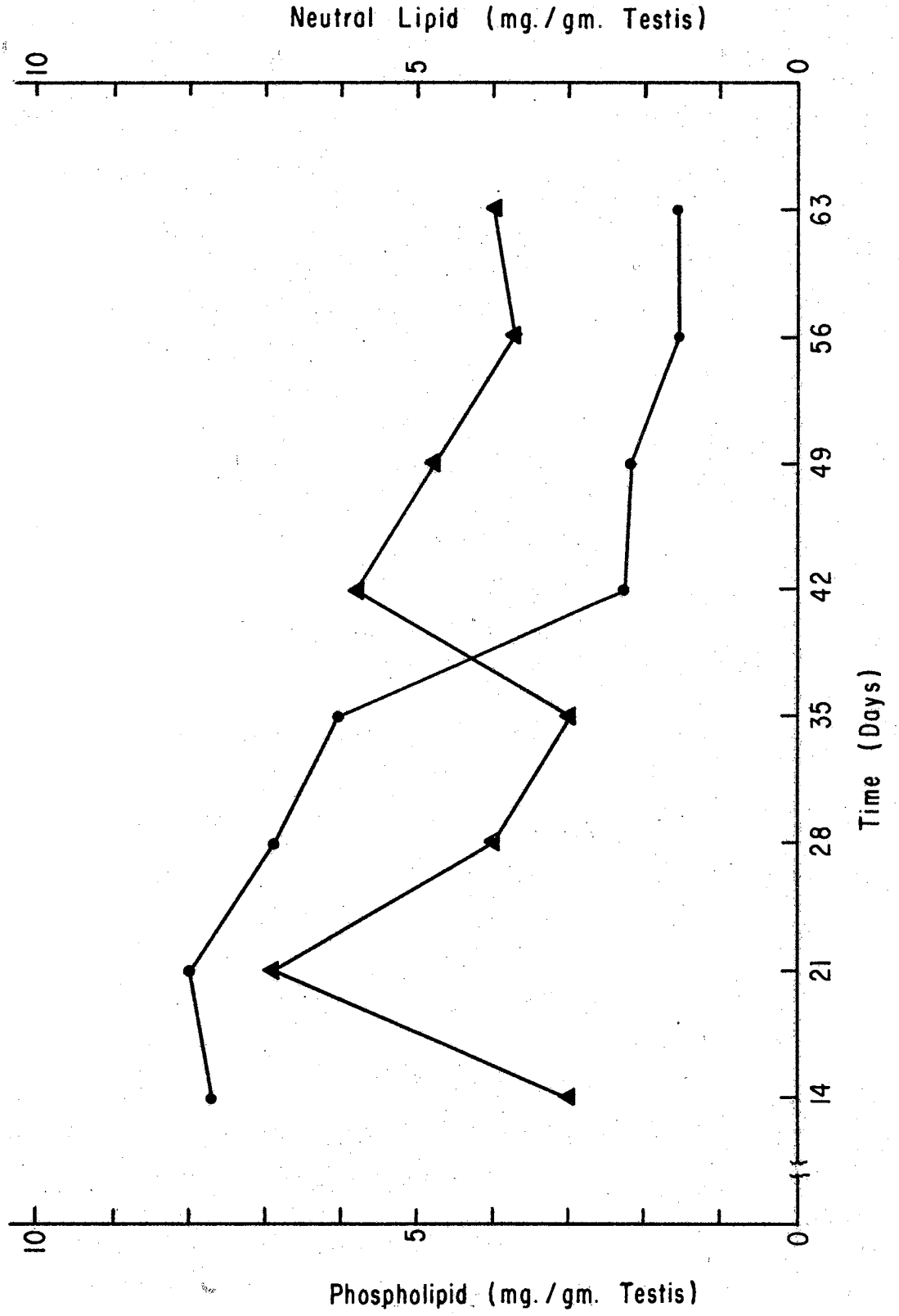

Figure 6. Phospholipid and Neutral Lipid Changes in the Testis of Control Rats During Postnatal Maturation. ▲, phospholipid; ●, neutral lipid.

ovary was less in a 4 week old animal than in an adult. Figure 6 does not indicate this to be true in the testis. The initial rise in phospholipid at 21 days appears to be a reflection of the corresponding increase in total lipid (Figure 5). Figure 5 shows that total lipid decreases continually from day 21 as the testis matures. This is reflected in Figure 6 by observing that neutral and phospholipid values decrease from day 21 with the exception of another phospholipid peak at 42 days. This rise may be explained by the observation of Clermont and Perey (17) that mature sperm cells are first seen in the rat testis at 35 days of age. It seems possible that the seminiferous tubules are syncronized at this time and that spermatozoa are being produced at the same rate in all of them, hence the rise in phospholipid. Hartree and Mann (34) in 1959 and Mann (54) in 1960 reported that phospholipids are present in high concentrations in ram and bull spermatozoa. The subsequent drop in phospholipid between 42-56 days could be explained by the first wave of sperm cells moving out of the testis and into the epididymis. Clegg (11) determined the age of fertility in rats to be 63 days and found that sperm require approximately 30 days in the epididymis before they appear in the vas deferens. The tubules may begin to function independently after the production of the initial sperm cells. Spermatozoa would, therefore, mature and move out of the testis at different times. This latter hypothesis is suggested by Clermont and Leblond (16) and Clermont (15) in the "Stem Cell Renewal Theory."

It should be kept in mind that the changes in phospholipid and neutral lipid are only relative to the total lipid values. Note in Figure 6 that neutral lipids decline in as sharp a manner as phospholipids increase between 35 and 42 days. The general trend of the neutral lipids is the same as that of the total lipid. Neutral lipids are composed of two main fractions: glycerides and sterols. Because of the different chemical nature of these groups of compounds they were separated by saponification. The saponifiable fraction contained fatty acids and glycerol, while sterols comprised the unsaponifiable portion.

Figure 7 shows the changes in concentration which occurred in the saponifiable and unsaponifiable lipids during testicular matura-

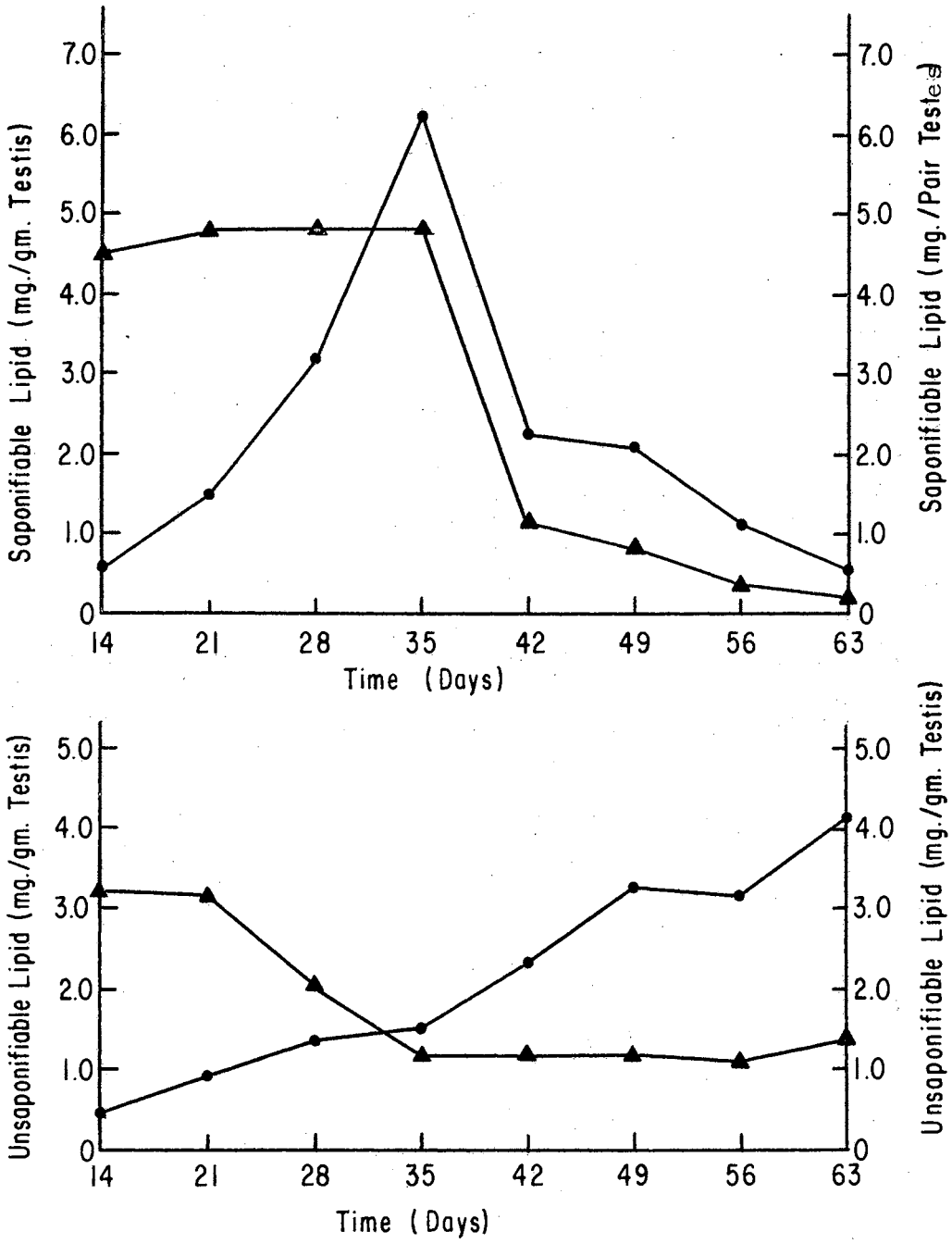

Figure 7. Changes in Saponifiable and Unsaponifiable Lipid Fractions of the Testis of Control Rats During Postnatal Maturation. ▲, mg./gm. testis; ●, mg./pair testes.

tion. The unsaponifiable fraction decreases from 21-35 days when expressed as mg. per gm. of testis, while the saponifiable portion remains essentially constant. This indicates that the decrease in neutral lipid over the same time period is reflected entirely in the sterols. From day 35 on, the unsaponifiable fraction seems to be maintained at a constant rate, while the saponifiable lipids decline sharply between 35 and 42 days. This shows that the 35-42 day drop in neutral lipid is due entirely to a decrease in glycerides. These results would indicate that from 21-35 days there occurs an increased catabolism or decreased synthesis of the sterols and from 35-42 days an increased breakdown or utilization of the glycerides. The latter may provide fatty acids and glycerol for the increased synthesis of phospholipids which appears to occur during the 35-42 day period. One might test this hypothesis by incubating testis slices in vitro with C^{14}-labeled triglycerides and isolating the phospholipids. The amount of C^{14} present in the phospholipid fraction would give some indication of synthesis from triglycerides. This, of course, would require much more conclusive evidence, since it is a well known fact that diglycerides are readily converted to phospholipids, and it is possible to form diglycerides from triglycerides by hydrolyzing one fatty acid.

The inorganic phosphate content of the total lipid and phospholipid fractions was determined (Appendix, p. 47). In all cases the inorganic phosphate content of the total lipids was higher than that of the phospholipids, indicating that the total lipid fraction contained some nonphospholipid phosphorous. The Kjeldahl nitrogen content of the phospholipids was also determined (Appendix, p. 48) and the results used to calculate the N:P molar ratio. This ratio was close to 1:1 in all cases, which should theoretically be the case, since there is 1 mole of nitrogen to 1 mole of phosphorous in most phospholipids.

The phospholipids seem to reach two peaks; one at 21 days and the other at 42. It would be interesting to separate the lipid fraction into the various types of phospholipids and observe which type or types of phospholipids are responsible for the overall change in phospholipid content. This could be accomplished by isolating the

phospholipid fraction and then employing thin layer chromatography, or silicic acid column chromatography to separate each type.

It would also be of interest to further investigate the composition of the saponifiable and unsaponifiable portions of the neutral lipid fraction. Since the saponifiable fraction is primarily fatty acids and the unsaponifiable portion is sterols, one could identify and quantitate the components of both fractions using gas-liquid chromatography.

In summary, then, the above data indicates that changes in biochemical constituents are occurring in the rat testis during postnatal development. The lipid content seems to reflect the greatest changes of the components investigated. The period between 35 and 42 days appears to be a critical period in testicular development because of the evident changes in lipid content. These changes are presumed to be due to the changing cell types and the formation of the first mature sperm cells at 35 days of age.

The results presented here have been collected from data derived from experiments with control scrotal testis. The biochemical changes occurring in the cryptorchid testis during maturation will now be presented. The data collected on these animals is presented in the appendix because of the predominant interest in the scrotal testis and because of lack of cryptorchid animals.

Cryptorchid Testis Experiments

The testes of the cryptorchid rats began to descend into the scrotum at 42 days, resulting in the lack of treated animals during the last three experimental weeks. Therefore, the cryptorchid data will only be considered between 21 and 42 days.

Body Weight and Testis Weight: The rats used for this section of the study were rendered bilaterally cryptorchid on the 14th day of age. The first data were collected when the treated animals were 21 days old. The cryptorchid rats gained weight each week, but to a lesser extent than did the control animals. Also the testis continued to grow in the abdominal cavity, but again the increase in size was much less than the increase evidenced in testis from control animals.

(Appendix, p. 49). Moore (63), Nelson (64), and Clegg (12, 13) found the same results in bilaterally cryptorchid rats. The excess heat of the body cavity is proposed to be the reason for the slower growth rate and subsequent atrophy of the experimentally retained testis.

Protein Content: The protein data followed the same trend as that from the control animals (Appendix, p. 49) except at an apparent level difference. The peak at 28 days again was noticed in the cryptorchid animals, which would indicate that protein synthesis may be stimulated, possibly by the gonadotropins. If these hormones do cause descent of the testis, these data indicate that the surge of gonadotropin is a function of age and is not a phenomenon restricted to untreated control testis.

Carbohydrate Content: The total carbohydrate data on the cryptorchid testis followed the same general trend as did the carbohydrate content of the control testis (Appendix, p. 50). The glycogen content of the treated testis decreased from 21-42 days (Appendix p. 50). This did not correspond with the control data and may indicate that Sertoli cells were not maturing in the cryptorchid testis. Clegg (13) in 1963 observed a degeneration of mature Sertoli cells in the experimentally retained testis of mature rats. This study also included histochemical data which indicated a decrease in testicular glycogen. It would be logical to assume that Sertoli cells do not mature in the immature cryptorchid testis, hence glycogen is not stored. Glycogen may also decrease due to an increased catabolism resulting in increased glucose formation.

Lipid Content: The total lipid, neutral lipid, phospholipid saponifiable, and unsaponifiable lipid fractions in the cryptorchid testis follow the same trends as do the corresponding fractions in the scrotal testis. The values do not appear to be appreciably different (Appendix p. 49). Nitrogen:phosphorous molar ratios were determined for the phospholipids and with the exception of the 42 day value, this ratio was very close to 1:1. In summary, the biochemical changes which occur in the cryptorchid testis during matura-

tion follow the same trends as the corresponding changes observed in control glands.

Thus, the data collected in this thesis indicate the following: (a) There are changes occurring in both scrotal and cryptorchid testis of the albino rat during maturation. The changes in both instances follow the same general trends; and (b) the most prevalent changes are those which occur in the lipid constituents of the scrotal testis. Total lipid and neutral lipid content decline during the period from 14-63 days of age, while two maxima are observed in the phospholipids at 21 and 42 days.

CHAPTER V

SUMMARY AND CONCLUSIONS

An experiment was designed to study biochemical changes occurring in scrotal and cryptorchid rat testis during postnatal development. One hundred twenty-eight 14-day-old male albino rats were obtained from the Holtzman Company. These animals were divided equally into 2 groups and 1 group was rendered bilaterally cryptorchid. Beginning on the 14th day, 5-8 animals from each group were sacrificed weekly for a period of 8 weeks. The testes were removed, stripped of fat, weighed, and pooled according to group.

The pooled tissue was analyzed for total protein, total carbohydrate, glycogen, and total lipid. The total lipid fraction was separated into neutral and phospholipids. The neutral lipid fraction was saponified into a saponifiable portion and an unsaponifiable fraction.

Changes were evidenced in all criteria investigated. The changes in cryptorchid testis followed the same trends as the changes observed in the scrotal testis, but there appeared to be a level difference. The cryptorchid values were lower in all cases. All the criteria measured showed a general increase when expressed in μg. or mg. per pair of testes. This general increase in biochemical constituents is concluded to be due to the general increase in testis size throughout the experiment.

Total protein and total carbohydrate showed an increase during maturation when expressed as mg. per gm. of testis. A very pronounced peak occurred on the protein graph at 28 days of age. This maximal value was not reflected on the paried testis line. If this is a true observation, it warrants further investigation. A logical first

step would be to assay for protein daily in a group of rats between 21 and 35 days old. This would pinpoint the time of the surge in protein content. An experiment could be designed to test the hypothesis that the peak is the result of increased protein synthesis. This might be accomplished by measuring the rate of incorporation of labeled amino acids into testicular protein. If protein synthesis is the cause of the peak the rate of incorporation of amino acids should be greatest in testis tissue derived from animals where the protein rise was observed.

With the exception of the protein peak, the most pronounced biochemical changes in the rat testis during maturation were observed in the lipid constituents of the control animals. Many areas of investigation are indicated by the lipid data. This study was designed to create questions concerning the biochemistry of the testis during maturation, therefore, conclusions can best be made by posing the following problems:

(1) The phospholipid fraction could be separated into the various types of phosphate containing lipids. This would indicate which type or types of phospholipids are responsible for the change in the total phospholipid fraction.

(2) The fatty acids present in the saponifiable fraction and the steroids which constitute the unsaponifiable lipids could be separated and identified. This mode of investigation would give some insight as to the specific compounds which cause the change in neutral lipids.

(3) The incorporation of C^{14}-labeled acetate into fatty acids or steroids could be studied. This would give some indication of the rate of synthesis of these compounds during maturation.

(4) The incorporation of C^{14}-labeled fatty acids into triglycerides or phospholipid would show the rate of turnover of these fractions.

(5) The rate of fatty acid oxidation could be determined. Homogenates, tissue slices, and subcellular fractions could be compared. This could be accomplished by measuring: (a) exogenous oxygen uptake, (b) the production of $C^{14}O_2$ from labeled fatty acids, (c) the reduction of a dye such as dichloroindophenol.

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APPENDIX

RAW DATA FROM CONTROL RATS

Age (days)	14	21	28	35	42	49	56	63
Ave. Body Wt. (gms.)	31.2	48.2	80.3	120.8	160.4	220.8	245.7	282.8
Ave. Testis Wt. (gms.)	0.1294	0.3008	0.6579	1.2678	1.9886	2.51	2.84	3.01
Total Lipid								
mg./gm. testis	12.15	14.22	11.00	9.2	7.72	6.86	5.5	5.76
mg./pair testes	1.57	4.28	7.24	11.66	15.35	17.22	15.62	17.3
Phospholipid								
mg./mg. total lipid	0.246	0.484	0.366	0.326	0.749	0.703	0.680	0.698
mg./gm. testis	2.99	6.88	4.03	3.00	5.78	4.82	3.74	4.02
mg./pair testes	0.39	2.1	2.65	3.8	11.5	12.1	10.62	12.1
Neutral Lipid								
mg./mg. total lipid	0.635	0.561	0.626	0.658	0.300	0.312	0.280	0.271
mg./gm. testis	7.72	7.98	6.89	6.05	2.32	2.14	1.54	1.56
mg./pair testes	1.00	2.37	4.53	7.67	4.61	5.37	4.37	4.70
Unsaponifiable								
mg./mg. neutral lipid	0.419	0.394	0.298	0.196	0.517	0.607	0.662	0.885
mg./gm. testis	3.23	3.14	2.05	1.19	1.20	1.30	1.13	1.38
mg./pair testes	0.42	0.94	1.35	1.51	2.29	3.26	3.21	4.15
Saponifiable								
mg./mg. neutral lipid	0.581	0.606	0.702	0.803	0.483	0.393	0.338	0.115
mg./gm. testis	4.49	4.84	4.84	4.86	1.12	0.84	0.41	0.18
mg./pair testes	0.58	1.46	3.18	6.16	2.23	2.11	1.16	0.54

RAW DATA FROM CONTROL RATS, Cont'd.

Age (days)	14	21	28	35	42	49	56	63
Total Carbohydrate								
mg./gm. testis	465	1089	1125	1215	1129	1215	1182	1482
mg./pair testes	60.17	327.6	740.1	1540.4	2245.1	3049.1	3356.9	4460.8
Total Protein								
mg./gm. testis	63.75	68.75	85.5	72.5	75.5	80.0	82.5	78.5
mg./pair testes	8.25	20.68	56.25	91.92	150.14	200.8	234.3	236.3
Glycogen of Lipid Free Extract								
mg./gm. L. F. extract	7.768	4.670	6.744	9.560	8.40	9.10	8.02	8.46
µg./gm. testis	875.2	623.1	772.9	990.0	807.5	923	783.2	850
µg./pair testes	113.3	187.4	508.5	627.6	1605.8	2339.2	2223.7	2558.5
iP* of Total Lipid								
µg./mg. Total lipid	14.0	11.8	11.3	12.0	25.5	24.3	36.9	23.6
µg./gm. testis	170.1	167.8	124.3	110.4	196.9	166.7	203.0	136
µg./pair testes	22.0	50.5	81.8	140	391.6	418.4	576.5	409.4
iP* of Phospholipid								
µg./mg. Total lipid	9.85	6.4	7.45	8.25	17.4	17.5	18.2	16.7
µg./gm. testis	119.7	91.0	82.0	75.9	134.3	120.0	100.1	96.0
µg./pair testes	15.5	27.4	53.9	96.2	267.0	301.2	284.3	289.0
Plasmalogen								
µm./gm. testis	13.7	12.1	12.46	7.0	5.92	5.38	10.2	8.1
µm./pair testes	1.77	3.64	8.20	8.87	11.77	13.50	28.97	24.4

RAW DATA FROM CONTROL RATS, Cont'd.

Age (days)	14	21	28	35	42	49	56	63
Kjeldahl N_2 of p-lipid								
µg./mg. Total lipid	2.8	2.8	3.9	3.6	9.9	7.7	8.5	6.2
µg./gm. testis	33.8	40.0	49.0	32.4	76.8	52.8	46.8	35.5
µg./pair testes	4.4	12.0	32.2	41.1	152.7	132.5	132.9	106.9
N:P molar ratio	0.65	1.04	1.35	0.97	1.31	1.00	1.06	0.83

*Inorganic Phosphate

RAW DATA FROM CRYPTORCHID RATS

Age (days)	21	28	35	42	49	56	63
Ave. Body Wt. (gms.)	46.2	80.9	105.2	145.2	198.7		292.2
Ave. Testis Wt. (gms.)	0.2929	0.5646	0.863	1.491	1.062		1.2705
Total Lipid							
mg./gm. testis	15.8	14.8	7.7	4.76	10.5		14.0
mg./pair testes	4.63	8.36	6.65	7.10	11.15		17.8
Phospholipid							
mg./mg. total lipid	0.391	0.361	0.120	0.595	0.554		0.512
mg./gm. testis	6.18	5.34	0.92	2.83	5.82		7.17
mg./pair testes	1.81	3.01	0.79	4.22	6.18		9.11
Neutral Lipid							
mg./mg. neutral lipid	0.605	0.605	0.860	0.410	0.446		0.425
mg./gm. testis	9.56	8.95	6.62	1.95	4.68		5.95
mg./pair testes	2.80	5.05	5.71	2.91	4.97		7.56
Unsaponifiable							
mg./mg. neutral lipid	0.419	0.318	0.215	0.634	0.596		0.906
mg./gm. testis	4.00	2.85	1.42	1.24	2.79		5.39
mg./pair testes	1.17	1.61	1.23	1.85	2.96		6.85
Saponifiable							
mg./mg. neutral lipid	0.581	0.682	0.784	0.366	0.404		0.094
mg./gm. testis	5.56	6.10	5.20	0.71	1.89		0.56
mg./pair testes	1.75	3.44	4.49	1.06	2.01		0.71

RAW DATA FROM CRYPTORCHID RATS, Cont'd.

Age (days)	21	28	35	42	49	56	63
Total Carbohydrate							
µg./gm. testis	855	750	1020	1095	1245		960
µg./pair testes	250.4	423.4	880.3	1632.6	1322.2		1219.7
Total Protein							
mg./gm. testis	72.5	80.25	66.0	73.5	68.5		70.5
mg./pair testes	21.2	45.3	57.0	109.6	72.8		89.6
Glycogen of Lipid Free Extract							
mg./gm. L. F. ext.	5.66	7.83	9.80	8.47	9.90		11.8
µg./gm. testis	990.1	915	887.1	843.0	990.1		1097.6
µg./pair testes	290.0	536.4	765.6	1256.9	1051.5		1394.5
iP* of Total lipid							
µg./mg. Total lipid	10.2	10.1	2.84	20.9	18.3		14.7
µg./gm. testis	161.8	149.5	21.9	99.5	192.2		20.5
µg./pair testes	47.5	84.4	18.9	148.4	204.1		260.5
iP* of Phospholipid							
µg./mg. Total lipid	6.2	6.84	2.1	15.0	13.7		10.9
µg./gm. testis	97.96	101.2	16.2	71.4	143.9		151.8
µg./pair testes	28.7	57.1	14.0	106.5	152.8		192.9
Plasmalogen							
µm./gm. testis	14.3	9.85	2.2	3.7	11.8		13.8
µm./pair testes	4.2	5.6	1.9	5.5	12.5		17.5

RAW DATA FROM CRYPTORCHID RATS, Cont'd.

Age (days)	21	28	35	42	49	56	63
Kjeldahl N_2 of p-lipid							
µg./mg. Total lipid	2.6	3.0	0.7	17.1	6.1		5.2
µg./gm. testis	41.3	45.0	5.4	81.5	64.2		71.9
µg./pair testes	12.1	25.4	4.7	147.0	68.2		91.3
N:P molar ratio	0.97	1.00	0.76	2.64	1.02		1.09

*Inorganic Phosphate

VITA

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